

Lot No.: **56E**

Lot-specific information

## **Olerup SSP<sup>®</sup> HLA-Cw\*04**

**Product number:** 101.612-12 – including *Taq* polymerase  
101.612-12u – without *Taq* polymerase

**Lot number:** 56E

**Expiry date:** 2010-May-01

**Number of tests:** 12

**Number of wells per test:** 24

**Storage - pre-aliquoted primers:** dark at -20°C

- PCR Master Mix: -20°C
- Adhesive PCR seals: RT
- Product Insert: RT

**This Product Description is only valid for Lot No. 56E.**

### **CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP<sup>®</sup>* HLA-Cw\*04 LOT**

The HLA-Cw\*04 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP<sup>®</sup>* HLA-Cw\*04 lot was made (**Lot No. X77**).

The primers of the wells detailed below has been exchanged or modified.

Well	5'-primer	3'-primer	rationale
4	-	-	Changed internal positive control primer pair to decrease primer dimer formation tendency.
5	Modified	-	Increased specificity of specific primer pair.
7	Added	Added	Primer pair added for the Cw*0432 allele.
9	-	Added	Primer added for the Cw*0430 allele.
10	Added	Added	Primer pair added for the Cw*0431 allele.
12	Added	Added	Primer pair added for the Cw*0430 allele.
15	Added	Added	Primer pair added for the Cw*0428 allele.
20	Exchanged	Exchanged	Increased resolution.

Changes in revision R01 compared to R00:

1. The Cw\*040101-040102, Cw\*0406 and Cw\*0413 alleles are faintly amplified by primer mix 5.

Changes in revision R02 compared to R01:

1. The Cw\*0408 allele is weakly amplified by primer mix 8.
2. The numbering of the foot notes in the specificity table has been corrected.

## PRODUCT DESCRIPTION

### HLA-Cw\*04 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw\*0401 to HLA-Cw\*0432 alleles.

#### PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

The 24 well cut PCR plate is marked with 'Cw\*04'.

Well No. 1 is marked with the Lot No. '56E'.

The PCR plates are heat-sealed with a PCR-compatible foil.

**Please note:** When removing each 24 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

#### INTERPRETATION

The interpretation of HLA-Cw\*04 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1, 4, 5, 7, 8, 11, 14 and 16 amplify non-HLA-Cw\*04 alleles. In addition, the B\*5802 allele will be amplified by primer mixes 5 and 8.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-C\*04 alleles, i.e. **Cw\*0401 to Cw\*0432**, recognized by the HLA Nomenclature Committee in April 2008<sup>1</sup> will give rise to unique amplification patterns by the primers in the HLA-Cw\*04 subtyping kit.

The HLA-Cw\*04 subtyping kit cannot distinguish the Cw\*04010101 to Cw\*040105 alleles and the Cw\*040401 and Cw\*040402 alleles.

<sup>1</sup>HLA-Cw alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

#### RESOLUTION IN HOMO- AND HETEROZYGOTES

The 29 HLA-Cw\*04 alleles give rise to 30 different amplification patterns that can be combined in 465 homozygous and heterozygous combinations. 254 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR product generated by primer mix 9, 10, 12, 14 and 15 were not considered in these calculations.

+++++---	---+---+	-----	0403,0413 = 0406,0418
+++++---	---+---	-----	0401,0406 = 0403,0404
+++++---	---+---+	-----	0403,0414 = 0403,0428
+++-+--	---+---+	-----	0407,0413 = 0413,0427 = 0413,0432
+++-+--	---+---	-----	0404,0407 = 0404,0427 = 0404,0432

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+++---+ -++---+ -	0410,0413 = 0413,0431
+++---+ -++---+ -	0404,0410 = 0404,0431
+++---+ -++---+ -	0401,0413 = 0404,0416 = 0404,0418 = 0413,0416 = 0413,0418
+++---+ -++---+ -	0405,0407 = 0405,0427 = 0405,0432
+++---+ -++---+ -	0405,0410 = 0405,0431
+++---+ -++---+ -	0405,0416 = 0405,0418
+++---+ -++---+ -	0405,0414 = 0405,0428
+++---+ -++---+ -	0401,0405 = 0405,0405
+++---+ -++---+ -	0407,0408 = 0408,0427 = 0408,0432
+++---+ -++---+ -	0407,0430 = 0427,0430 = 0430,0432
+++---+ -++---+ -	0407,0409N = 0409N,0427 = 0409N,0432
+++---+ -++---+ -	0407,0411 = 0411,0432
+++---+ -++---+ -	0407,0410 = 0407,0431 = 0410,0427 = 0410,0432 = 0427,0431 = 0431,0432
+++---+ -++---+ -	0407,0429 = 0427,0429 = 0429,0432
+++---+ -++---+ -	0407,0412 = 0412,0427 = 0412,0432
+++---+ -++---+ -	0407,0418 = 0416,0432 = 0418,0427 = 0418,0432
+++---+ -++---+ -	0407,0414 = 0407,0428 = 0414,0427 = 0414,0432 = 0427,0428 = 0428,0432
+++---+ -++---+ -	0407,041501 = 041501,0427 = 041501,0432
+++---+ -++---+ -	0407,0417 = 0417,0427 = 0417,0432
+++---+ -++---+ -	0407,0419 = 0419,0427 = 0419,0432
+++---+ -++---+ -	0407,0420 = 0420,0427 = 0420,0432
+++---+ -++---+ -	0407,041502 = 041502,0427 = 041502,0432
+++---+ -++---+ -	0407,0423 = 0423,0427 = 0423,0432
+++---+ -++---+ -	0407,0424 = 0424,0427 = 0424,0432
+++---+ -++---+ -	0407,0425 = 0425,0427 = 0425,0432
+++---+ -++---+ -	0407,0426 = 0426,0427 = 0426,0432
+++---+ -++---+ -	0401,0407 = 0401,0427 = 0401,0432 = 0407,0432 = 0427,0432 = 0432,0432
+++---+ -++---+ -	0408,0410 = 0408,0431
+++---+ -++---+ -	0408,0416 = 0408,0418
+++---+ -++---+ -	0408,0414 = 0408,0428
+++---+ -++---+ -	0401,0408 = 0408,0408
+++---+ -++---+ -	0409N,0411 = 0410,0430 = 0411,0430 = 0430,0431
+++---+ -++---+ -	0409N,0410 = 0409N,0431
+++---+ -++---+ -	0416,0430 = 0418,0430
+++---+ -++---+ -	0414,0430 = 0428,0430
+++---+ -++---+ -	0401,0430 = 0409N,0429 = 0409N,0430 = 0429,0430 = 0430,0430
+++---+ -++---+ -	0409N,0416 = 0409N,0418
+++---+ -++---+ -	0409N,0414 = 0409N,0428
+++---+ -++---+ -	0401,0409N = 0409N,0409N
+++---+ -++---+ -	0411,0414 = 0411,0428
+++---+ -++---+ -	0401,0411 = 0410,0429 = 0411,0429 = 0411,0431 = 0429,0431
+++---+ -++---+ -	0410,0412 = 0412,0431
+++---+ -++---+ -	0410,0418 = 0416,0431 = 0418,0431
+++---+ -++---+ -	0410,0414 = 0410,0428 = 0414,0431 = 0428,0431
+++---+ -++---+ -	0410,041501 = 041501,0431
+++---+ -++---+ -	0410,0417 = 0417,0431
+++---+ -++---+ -	0410,0419 = 0419,0431
+++---+ -++---+ -	0410,0420 = 0420,0431
+++---+ -++---+ -	0410,041502 = 041502,0431
+++---+ -++---+ -	0410,0423 = 0423,0431

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+++----- -++----- -----+--	0410,0424 = 0424,0431
+++----- -++----- -----+--	0410,0425 = 0425,0431
+++----- -++----- -----+--	0410,0426 = 0426,0431
+++----- -++----- -----	0401,0410 = 0401,0431 = 0410,0431 = 0431,0431
+++----- --++-+-- -----	0416,0429 = 0418,0429
+++----- --++-+-- -----	0414,0429 = 0428,0429
+++----- --++-+-- -----	0401,0429 = 0429,0429
+++----- --++-+-- -----	0412,0416 = 0412,0418
+++----- --++-+-- -----	0412,0414 = 0412,0428
+++----- --++-+-- -----	0401,0412 = 0412,0412
+++----- --++-+-- -----	0414,0416 = 0414,0418 = 0416,0428 = 0418,0428
+++----- --++-+-- -----	041501,0416 = 041501,0418
+++----- --++-+-- +--+-----	0416,0417 = 0417,0418
+++----- --++-+-- +--+-----	0416,0419 = 0418,0419
+++----- --++-+-- +--+-----	0416,0420 = 0418,0420
+++----- --++-+-- +--+-----	041502,0416 = 041502,0418
+++----- --++-+-- -----+--	0416,0423 = 0418,0423
+++----- --++-+-- -----+--	0416,0424 = 0418,0424
+++----- --++-+-- -----+--	0416,0425 = 0418,0425
+++----- --++-+-- -----+--	0416,0426 = 0418,0426
+++----- --++-+-- -----	0401,0416 = 0401,0418 = 0416,0418 = 0418,0418
+++----- --++-+-- -----	0414,041501 = 041501,0428
+++----- --++-+-- +--+-----	0414,0417 = 0417,0428
+++----- --++-+-- +--+-----	0414,0419 = 0419,0428
+++----- --++-+-- +--+-----	0414,0420 = 0420,0428
+++----- --++-+-- +--+-----	0414,041502 = 041502,0428
+++----- --++-+-- +--+-----	0414,0423 = 0423,0428
+++----- --++-+-- +--+-----	0414,0424 = 0424,0428
+++----- --++-+-- +--+-----	0414,0425 = 0425,0428
+++----- --++-+-- +--+-----	0414,0426 = 0426,0428
+++----- --++-+-- -----	0401,0414 = 0401,0428 = 0414,0428 = 0428,0428
+++----- --++-+-- -----	0401,041501 = 041501,041501
+++----- --++-+-- +--+-----	0401,0417 = 041502,0417 = 0417,0417
+++----- --++-+-- +--+-----	0401,0419 = 0419,0419
+++----- --++-+-- +--+-----	0401,0420 = 0420,0420
+++----- --++-+-- +--+-----	0401,041502 = 041502,041502
+++----- --++-+-- +--+-----	0401,0423 = 0423,0423
+++----- --++-+-- +--+-----	0401,0424 = 0424,0424
+++----- --++-+-- +--+-----	0401,0425 = 0425,0425
+++----- --++-+-- +--+-----	0401,0426 = 0426,0426
++-++-+-- --+----- -----	0406,0407 = 0406,0427
++-++-+-- --+----- -----	0403,0407 = 0403,0427
++-++-+-- --+----- -----	0407,0416 = 0416,0427
++-++-+-- --+----- -----	0407,0407 = 0407,0427
-++-+-- --+----- -----	0404,0413 = 0413,0413

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## SPECIFICITY TABLE

### HLA-Cw\*04 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-Cw\*04 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-Cw*04 alleles	Other amplified HLA Class I alleles <sup>3</sup>
1	250 bp	800 bp	04010101-040105, 0403, 0405, 0407-0412, 041501-0418, 0419 <sup>weakly</sup> , 0420, 0423-0432	010201-0120, 020201-020203, 020205-020206, 0204-0215, 0217, 0219-0221, 050101-0510, 0512-0516, 0518, 0519, 06020101-0603, 0605-0617, 0802, 0805, 0807, 0812, 0815, 120201-1213, 1215-1217, 1221, 140201-1405, 1407N-1409, 1508, 160401, 1801-1803
2	220 bp	1070 bp	04010101-040105, 040401-0405, 0407-0409N, 0412 <sup>weakly</sup> , 0413-041502, 0417-0420, 0423-0432	
3 <sup>4</sup>	145 bp	1070 bp	04010101-040105, 040401-0405, 0408-041502, 0417-0420, 0423-0426, 0428-0432	
4 <sup>5</sup>	210 bp	1070 bp	0403, 0406	0212
5	250 bp	1070 bp	040401-040402 <sup>faintly</sup> , 0406 <sup>faintly</sup> , 0413 <sup>faintly</sup>	0203, 0216, 0218, 0511, 0517, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813,

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				0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1521, 1701- 1704, <b>B*5802</b>
<b>6<sup>4</sup></b>	95 bp	1070 bp	0405	
<b>7<sup>4</sup></b>	145 bp	1070 bp	0407, 0427, 0432	0334, 1803
<b>8</b>	270 bp	1070 bp	0408 <sup>weakly</sup>	1515, <b>B*5802</b>
<b>9<sup>6,7,8</sup></b>	180, 220 bp	1070 bp	0409N, 0430	
<b>10<sup>9</sup></b>	195, 220 bp	1070 bp	0410, 0411, 0431	
<b>11</b>	180 bp	1070 bp	04010101- 040105, 0403- 0410, 0412- 0420, 0423- 0426, 0428- 0432	0605
<b>12<sup>4,10</sup></b>	125, 180 bp	1070 bp	0411, 0429, 0430	
<b>13</b>	225 bp	1070 bp	0412	
<b>14<sup>11</sup></b>	155, 190 bp	1070 bp	0413, 0416, 0418	0708
<b>15<sup>6,12</sup></b>	170, 190 bp	1070 bp	0414, 0428	
<b>16<sup>4</sup></b>	130 bp	1070 bp	041501	0305, 0325, 0327, 1409
<b>17<sup>4,6</sup></b>	90 bp	<b>800 bp</b>	0417	
<b>18</b>	225 bp	1070 bp	0419	
<b>19</b>	155 bp	1070 bp	0420	
<b>20</b>	540 bp	1070 bp	041502, 0417	
<b>21<sup>4</sup></b>	85 bp	1070 bp	0423	
<b>22<sup>4</sup></b>	130 bp	1070 bp	0424	
<b>23<sup>4</sup></b>	85 bp	1070 bp	0425	
<b>24</b>	170 bp	1070 bp	0426	

<sup>1</sup>Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-Cw\*04 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

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PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw\*04 subtyping.

In addition, well number 17 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-Cw alleles some non-HLA-Cw\*04 alleles will be amplified by primer mixes 1, 4, 5, 7, 8, 11, 14 and 16.

<sup>4</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup>Primer mix 4 may give rise to primer dimer formation.

<sup>6</sup>The primers in primer mixes 9, 15 and 17 have a tendency of giving rise to nonspecific amplifications.

<sup>7</sup>The specific PCR product generated by the primers in primer mix 9 may be less intense than the other Cw\*04 specific PCR products.

<sup>8</sup>Primer mix 9: Specific PCR fragment of 180 bp in the Cw\*0430 allele. Specific PCR fragment of 220 bp in the Cw\*0409N allele.

<sup>9</sup>Primer mix 10: Specific PCR fragment of 195 bp in the Cw\*0431 allele. Specific PCR fragment of 220 bp in the Cw\*0410 and Cw\*0411 alleles.

<sup>10</sup>Primer mix 12: Specific PCR fragment of 125 bp in the Cw\*0411 and Cw\*0429 alleles. Specific PCR fragment of 180 bp in the Cw\*0430 allele.

<sup>11</sup>Primer mix 14: Specific PCR fragment of 155 bp in the Cw\*0416 allele. Specific PCR fragment of 190 bp in the Cw\*0413, Cw\*0418 and Cw\*0708 alleles.

<sup>12</sup>Primer mix 15: Specific PCR fragment of 170 bp in the Cw\*0414 allele. Specific PCR fragment of 190 bp in the Cw\*0428 allele.

<b>INTERPRETATION TABLE</b>												
<b>HLA-Cw*04 SSP subtyping</b>												
Amplification patterns of the Cw*0401 to Cw*0432 alleles												
	Well <sup>5</sup>											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	250	220	145	210	250	95	145	270	180	195	180	125
PCR product									220	220		180
Length of int.	<b>800</b>	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
pos. control <sup>1</sup>												
5'-primer(s) <sup>2</sup>	2 <sup>nd</sup>	112	112	118	2 <sup>nd</sup>	98	112	2 <sup>nd</sup>	1018	112	201	218
	5'-CCA <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-gTg <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-ggA <sup>3'</sup>
							459			368		1017
							5'-gAT <sup>3'</sup>			5'-gTT <sup>3'</sup>		5'-gTg <sup>3'</sup>
3'-primer(s) <sup>3</sup>	539	289	218	289	539	154	218	559	1052	289	341	302
	5'-TCC <sup>3'</sup>	5'-AgC <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-AgC <sup>3'</sup>	5'-TCA <sup>3'</sup>	5'-CAG <sup>3'</sup>	5'-gCg <sup>3'</sup>	5'-CAG <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-AgT <sup>3'</sup>	5'-CgT <sup>3'</sup>	5'-ggC <sup>3'</sup>
							559		1092	521		1052
							5'-CTC <sup>3'</sup>		5'-TTA <sup>3'</sup>	5'-ggA <sup>3'</sup>		5'-Tgg <sup>3'</sup>
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-Cw allele <sup>4</sup>												
*04010101-040105	1	2	3								11	
*0403	1			4							11	
*040401-040402		2	3		f						11	
*0405	1	2	3			6					11	
*0406				4	f						11	
*0407	1	2					7				11	
*0408	1	2	3					w			11	
*0409N	1	2	3						9		11	
*0410	1		3							10	11	
*0411	1		3							10		12
*0412	1	w	3								11	
*0413		2	3		f						11	
*0414		2	3								11	
*041501	1	2	3								11	
*041502	1	2	3								11	
*0416	1										11	
*0417	1	2	3								11	
*0418	1	2	3								11	
*0419	w	2	3								11	
*0420	1	2	3								11	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12



INTERPRETATION TABLE												
HLA-Cw*04 SSP subtyping												
Amplification patterns of the Cw*0401 to Cw*0432 alleles												
Well <sup>5</sup>												
13	14	15	16	17	18	19	20	21	22	23	24	
225	155	170	130	90	225	155	540	85	130	85	170	Length of spec. PCR product
	190	190										
1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	Length of int. pos. control <sup>1</sup>
112	105	412	369	2 <sup>nd</sup> I	368	347	112	368	127	172	89	5'-primer <sup>2</sup>
5'-CCT <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-ATA <sup>3'</sup>	5'-TAC <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-gTA <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-ggA <sup>3'</sup>	5'-TCC <sup>3'</sup>	5'-gAT <sup>3'</sup>	
	368	697										
	5'-gTT <sup>3'</sup>	5'-TCC <sup>3'</sup>										
295	218	539	459	379	550	459	368	412	218	218	218	3'-primer <sup>3</sup>
5'-TCC <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-TCT <sup>3'</sup>	5'-AgA <sup>3'</sup>	5'-CAC <sup>3'</sup>	5'-CAT <sup>3'</sup>	5'-AgA <sup>3'</sup>	5'-CAT <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-gCT <sup>3'</sup>	
	514	846										
	5'-CTT <sup>3'</sup>	5'-CAC <sup>3'</sup>										
	527											
	5'-CCg <sup>3'</sup>											
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												HLA-Cw allele <sup>4</sup>
												*04010101-040105
												*0403
												*040401-040402
												*0405
												*0406
												*0407
												*0408
												*0409N
												*0410
												*0411
13												*0412
	14											*0413
		15										*0414
			16									*041501
							20					*041502
	14											*0416
				17			20					*0417
	14											*0418
					18							*0419
						19						*0420
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **56E**

Lot-specific information

Length of spec.	250	220	145	210	250	95	145	270	180	195	180	125
PCR product									220	220		180
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0423	1	2	3								11	
*0424	1	2	3								11	
*0425	1	2	3								11	
*0426	1	2	3								11	
*0427	1	2					7					
*0428	1	2	3								11	
*0429	1	2	3								11	12
*0430	1	2	3						9		11	12
*0431	1	2	3							10	11	
*0432	1	2	3				7				11	
*010201-0120, 020201-020203, 020205-020206, 0204-0211, 0213-0215, 0217, 0219-0221, 050101-050104, 0503-0510, 0512-0516, 0518, 0519, 06020101-0603, 0606-0617, 0802, 0805, 0807, 0812, 0815, 120201-1213, 1215-1217, 1221, 140201-1404, 1407N, 1408, 1508, 160401, 1801, 1802	1											
*0203, 0216, 0218, 0511, 0517, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1513, 1516-1521, 1701-1704					5							
*0212	1			4								
*0305, 0325, 0327												
*0334							7					
*0605	1										11	
*0708												
*1409	1											
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **56E**

Lot-specific information

225	155	170	130	90	225	155	540	85	130	85	170	Length of spec. PCR product Well No.
13	14	15	16	17	18	19	20	21	22	23	24	
								21				*0423
									22			*0424
										23		*0425
											24	*0426
												*0427
		15										*0428
												*0429
												*0430
												*0431
												*0432
												*010201-0120, 020201-020203, 020205-020206, 0204-0211, 0213-0215, 0217, 0219-0221, 050101-050104, 0503-0510, 0512-0516, 0518, 0519, 06020101-0603, 0606-0617, 0802, 0805, 0807, 0812, 0815, 120201-1213, 1215-1217, 1221, 140201-1404, 1407N, 1408, 1508, 160401, 1801, 1802
												*0203, 0216, 0218, 0511, 0517, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 0816, 1214, 1218, 1220, 1406, 150201-1507, 1509-1513, 1516-1521, 1701-1704
												*0212
			16									*0305, 0325, 0327
												*0334
												*0605
	14											*0708
			16									*1409
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **56E**

Lot-specific information

<b>Length of spec.</b>	<b>250</b>	<b>220</b>	<b>145</b>	<b>210</b>	<b>250</b>	<b>95</b>	<b>145</b>	<b>270</b>	<b>180</b>	<b>195</b>	<b>180</b>	<b>125</b>
<b>PCR product</b>									<b>220</b>	<b>220</b>		<b>180</b>
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>*1515</b>					<b>5</b>			<b>8</b>				
<b>HLA-Cw allele<sup>4</sup></b>												
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>B*5802</b>					<b>5</b>	<b>8</b>						
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw\*04 subtyping. .

In addition, well number 17 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

<sup>2</sup>The nucleotide position, in the 2<sup>nd</sup>, 3<sup>rd</sup> or 6<sup>th</sup>, exon or in the 2<sup>nd</sup> intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup>, 3<sup>rd</sup> or 7<sup>th</sup> exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

Lot No.: **56E**

Lot-specific information

225	155	170	130	90	225	155	540	85	130	85	170	Length of spec. PCR product
	190	190										Well No.
13	14	15	16	17	18	19	20	21	22	23	24	*1515
												HLA-Cw allele <sup>4</sup>
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												B*5802
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

<sup>4</sup>The nucleotide sequence of the Cw\*0402 allele has been shown to be identical to Cw\*04010101.

The nucleotide sequence of the Cw\*0421 allele has been renamed to Cw\*041502.

The nucleotide sequence of the Cw\*0422 allele has been shown to be identical to Cw\*0421.

<sup>5</sup>Primer mix 9: Specific PCR fragment of 180 bp in the Cw\*0430 allele. Specific PCR fragment of 220 bp in the Cw\*0409N allele.

Primer mix 10: Specific PCR fragment of 195 bp in the Cw\*0431 allele. Specific PCR fragment of 220 bp in the Cw\*0410 and Cw\*0411 alleles.

Primer mix 12: Specific PCR fragment of 125 bp in the Cw\*0411 and Cw\*0429 alleles. Specific PCR fragment of 180 bp in the Cw\*0430 allele.

Primer mix 14: Specific PCR fragment of 155 bp in the Cw\*0416 allele. Specific PCR fragment of 190 bp in the Cw\*0413, Cw\*0418 and Cw\*0708 alleles.

Primer mix 15: Specific PCR fragment of 170 bp in the Cw\*0414 allele. Specific PCR fragment of 190 bp in the Cw\*0428 allele.

'w', may be weakly amplified.

'f', may be faintly amplified.

Lot No.: **56E**

Lot-specific information

<b>CELL LINE VALIDATION SHEET</b>																				
<b>HLA-Cw*04 SSP subtyping kit</b>																				
				<b>Well</b>																
				<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	
				Prod. No.:	200618401	200618402	200618403	200846004	200846005	200618406	200846007	200618408	200846009	200846010	200618411	200846012	200733713	200733714	200846015	200618416
<b>IHW cell line</b>			<b>Cw*</b>																	
1	9001	SA	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1202		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
5	9009	KAS011	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0304	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0602		+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
37	9065	HHKB	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0202	*0701	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0602		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*0802		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*1701	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-

Lot No.: **56E**

Lot-specific information

CELL LINE VALIDATION SHEET												
HLA-Cw*04 SSP subtyping kit												
				Well								
				17	18	19	20	21	22	23	24	
				Prod. No.:	200733717	200733718	200733719	200846020	200733721	200733722	200733723	200733724
	IHWC cell line		Cw*									
1	9001	SA	*0702		-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	-	-	-	-	-	-	-	-
3	9011	E4181324	*1202		-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	-	-	-	-	-	-	-	-
5	9009	KAS011	*0602		-	-	-	-	-	-	-	-
6	9353	SM	*0304	*0702	-	-	-	-	-	-	-	-
7	9020	QBL	*0501		-	-	-	-	-	-	-	-
8	9007	DEM	*0602		-	-	-	-	-	-	-	-
9	9026	YAR	*1203		-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-
12	9052	DBB	*0602		-	-	-	-	-	-	-	-
13	9067	BTB	*0102		-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	-	-	-	-	-	-	-	-
15	9075	DKB	*0304		-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-
17	9008	WILJON	*1203		-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		-	-	-	-	-	-	-	-
21	9064	AMALA	*0303		-	-	-	-	-	-	-	-
22	9056	KOSE	*1203		-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1203		-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	-	-	-	-	-
31	9019	DUCAF	*0501		-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	-	-	-	-	-
33	9098	MT14B	*0304		-	-	-	-	-	-	-	-
34	9104	DHIF	*1203		-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	-	-	-	-	-	-	-	-
37	9065	HHKB	*0702		-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	-	-	-	-	-	-
39	9315	CML	*0202	*0701	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0602		-	-	-	-	-	-	-	-
41	9055	H0301	*0802		-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*1701	-	-	-	-	-	-	-	-
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	-	-	-	-	-	-	-	-

Lot No.: **56E**

Lot-specific information

## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA-Cw\*04 SSP**

**Product number:** 101.612-12 – including *Taq* polymerase  
101.612-12u – without *Taq* polymerase

**Lot number:** 56E

**Expiry date:** 2010-May-01

**Number of tests:** 12

**Number of wells per test:** 24

#### **Well specifications:**

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2006-184-01	9	2008-460-09	17	2007-337-17
2	2006-184-02	10	2008-460-10	18	2007-337-18
3	2006-184-03	11	2006-184-11	19	2007-337-19
4	2008-460-04	12	2008-460-12	20	2008-460-20
5	2008-460-05	13	2007-337-13	21	2007-337-21
6	2006-184-06	14	2007-337-14	22	2007-337-22
7	2008-460-07	15	2008-460-15	23	2007-337-23
8	2006-184-08	16	2006-184-16	24	2007-337-24

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 6, 7, 8, 10, 12, 13, 15, 17 to 19 to 24 were available. The specificity of the primers in primer solutions 7, 8, 10, 12, 15, 17, 20 and 21 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 13 and 18 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 19, 22, 23 and 24 it was only possible to test the 3'-primers, the 5'-primers were not possible to test.

**Results:** No false positive or false negative amplifications were obtained.

**Date of approval:** 2008-May-28

**Approved by:**

**Quality Control, Supervisor**



Lot No.: **56E**

Lot-specific information

## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-Cw\*04  
**Product number:** 101.612-12, 101.612-12u  
**Lot number:** 56E

**Intended use:** HLA-Cw\*04 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB  
Hasselstigen 1  
SE-133 33 Saltsjöbaden, Sweden  
**Phone:** +46-8-717 88 27  
**Fax:** +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2003, ISO 17025:1999 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III.

The Technical Construction File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Saltsjöbaden, Sweden  
2008-May-28

Olle Olerup  
Managing Director

101.612-12 – including *Taq* polymerase

101.612-12u – without *Taq* polymerase

Lot No.: **56E**

Lot-specific information

101.612-12 – including *Taq* polymerase

101.612-12u – without *Taq* polymerase

Lot No.: **56E**

Lot-specific information

HLA-Cw\*04  
101.612-12 – including *Taq* polymerase  
101.612-12u – without *Taq* polymerase

Product Insert

Page 20 of 20

Lot No.: **56E**

Lot-specific information

**ADDRESSES:**

**Manufacturer:**

**Olerup SSP AB**, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

**Tel:** +46-8-717 88 27

**Fax:** +46-8-717 88 18

**E-mail:** [info-ssp@olerup.com](mailto:info-ssp@olerup.com)

**Web page:** <http://www.olerup.com>

**Distributed by:**

**Olerup GmbH**, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

**Tel:** +43-1-710 15 00

**Fax:** +43-1-710 15 00 10

**E-mail:** [support-at@olerup.com](mailto:support-at@olerup.com)

**Web page:** <http://www.olerup.com>

**Olerup Inc.**, 901 S. Bolmar St., Suite R, West Chester, PA 19382

**Tel:** 1-877-OLERUP1

**Fax:** 610-344-7989

**E-mail:** [info.us@olerup.com](mailto:info.us@olerup.com)

**Web page:** <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.